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Original Article

Solvent Polarity from Different Plant Extracts Deticate the Potential Activity against Infection by Methicillin-Resistant *Staphylococcus aureus* in Saudi Arabia

Taha A. Kumosani[1,2,3,*], Sharifah H. Alshehri[1], Fatma A. Eissa[1], Elie K. Barbour[2,3], Soonham S. Yaghmoor[2,3], Turkyah J. Albogami[1], Sultan N. Sunble[1,2], Tarek A. Abdelnapy[4,5], Said S. Moselhy[6,*]

[1]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, SAUDI ARABIA.
[2]Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, SAUDI ARABIA.
[3]Production of Bio-Products for Industrial Application Research Group, King Abdulaziz University, Jeddah, SAUDI ARABIA.
[4]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, King Abdulaziz University, Jeddah, SAUDI ARABIA.
[5]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Cairo, EGYPT.
[6]Biochemistry Department, Faculty Of Science, Ain Shams University, Cairo, EGYPT.

ABSTRACT

Background: Skin infection by Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a common disease in Saudi Arabia. This bacterium is resistance to Methicillin and other antibiotics. Skin and soft tissue are the greatest common infected areas by these strains include abscesses, wound infections and other diseases. **Materials and Methods:** This study evaluated the *in vitro* antibacterial action of different plants extracts using different solvents from Saudi Arabia against MRSA. Different Plants were extracted by different solvents: methanol, chloroform and n-hexane. Agar well diffusion method was used to determine antibacterial efficacy with 100 µL (5 mg /0.5 mL) of plant extracts. **Results:** Minimum inhibitory concentration was determined on plant extracts that showed high effectiveness against MRSA. **Conclusion:** The obtained from Gas chromatography/mass spectrum analysis of the most active four plants, revealed the active volatile oils and flavonoids contents of *Artemisia monosperma, Mentha longifolia, Vernonia schimperi* and *Rumex nervosu* have antibacterial activity against MRSA. These plants demonstrate promising anti-MRSA potential.

Keywords: Methicillin-Resistant, *Staphylococcus aureus*, Solvent polarity, Plant extract.

Correspondence:
Prof. Taha A. Kumosani[1,2,3]
[1]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, SAUDI ARABIA.
[2]Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, SAUDI ARABIA.
[3]Production of Bio-Products for Industrial Application Research Group, King Abdulaziz University, Jeddah, SAUDI ARABIA.
Email: tkumosani@gmail.com

Prof. Said S. Moselhy
Department of Biochemistry, Faculty of Science, Ain shams University, Cairo, EGYPT.
Email: moselhy6@hotmail.com

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INTRODUCTION

Skin infection by Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a universal infection that arise resistant to methicillin and another β-lactam antibiotic that used to cure them.[1] MRSA is an economic nosocomial pathogen that causes serious morbidity and mortality worldwide.[2] The infection of this bacteria is not limited to patients who stay in hospitals or who have weak immune systems, but extends to other people such as sports teams, military recruits, or prisoners. It's called Community Associated MRSA (CA-MRSA) strains.[3] Skin and soft tissue are the greatest common infected areas by these strains, manifestations of infections in these sites include folliculitis, furuncles and carbuncles, impetigo, mastitis, wound infections and staphylococcal scalded skin syndrome.[4] Despite significant development in the manufacture of drugs and drugs prepared from pure chemicals, the number of microorganisms resistant to traditional antibiotics is increasing.[5] In different countries, medicinal plants are used as a source of many effective drugs and parts of some plants were used as extract for row medicines that have many of biological activities.[6] Herbal medicines are commonly prescribed because of their efficacy, their decreased side effects, and their low cost.[7] Many published papers suggested that medicinal plants with antibacterial activity can be considered for treatment of MRSA infections.[8] One of these plants used as therapy is *Artemisia monosperma* Del. It is a green aromatic plant growth in the northern area in Saudi Arabia.

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It is utilized in conventional medicine for the therapy of many illnesses among them bacterial infections.[9] *Lavandula dentata* L. is a usually distributed plant cultivated in the temperate climates. Previous research found the aerial parts in *Lavandula dentata* have good antimicrobial activity against *S. aureus*.[10] *Leptadenia pyrotechnica*, belonging to family Asclepiadaceae. This plant is growing in different parts of Africa, Asia and Mediterranean region, and known in the Arabic language as Markh. The plant is ethno-medicinally used for the treatment of wounds and some skin disease.[11] *Ficus vasta forssk.* is a very large plant rising up 25 m high. In previous studies showed the leaves have antioxidant and antimicrobial activity.[12] *Zizyphus spina-christi* has been reported to have activity against bacterial and fungal pathogens which are normally quite resistant to modern medications.[13] This study is designed to evaluate antibacterial activity *in vitro* of different plant extracts against MRSA.

MATERIALS AND METHODS

Plant samples

Plants were collected from different regions in the Kingdom of Saudi Arabia (Table 1). The collected plants were identified to the species and genus level, by the Plant Taxonomy Unit, Botany Department at King Abdulaziz University. The plants were washed to remove the soils and other particles, dry in a dark environment for three days, then grinded by a grinding machine (Alsaif-Elec E02300, Korea) until it becomes a powder, then labeled by genus, species, time and date of collection, plant part and region. Plants were saved in vacuum bags by vacuum sealer machine (Swiss Home, SH-6691, China) to prevent microbial growth and kept at -4°C until used.

Preparation of plant extract

Extraction molecules from plants to test their antibacterial activity against MRSA. The solvents used are methanol, chloroform and n-hexane (Fisher Chemical, Loughborough, UK). The method used is a reflux method that is preferable for plants extraction, pending the metabolites are heat stable. Plant samples (5 g) were flooded in different solvents (100 mL) for 24 hr in the water bath (Grant instrument, Cambridge) at 60°C. Mixtures were then filtered using the Whatman Filter paper No.1, then concentrated at 60°C by rotary evaporator (Dragon Lab, RE100-pro, China) and left dried in cabinet for 24 hr. The extracts were stored at 4°C until used.[14]

Methicillin-Resistant *S. aureus*

Bacteria were isolated and identified from King Abdulaziz University Hospital, Jeddah, KSA.

Antibacterial activity

Agar well diffusion technique was utilized to determine the antibacterial efficacy against MRSA.[15] The cultures were grown in Muller Hinton broth (NutriSelect® Plus. Germany) overnight. Final cell concentrations were adjusted to McFarland standard. After that, bacterial suspension was equally inoculated on the Mueller Hinton agar (NutriSelect® Plus. Germany) plates by sterile cotton swap. Then, the wells were performed in every plate by cork borer (Humboldt, H-9661, USA). The concentration of plants extracts was prepared 5 mg/0.5 mL by dimethyl sulfoxide (DMSO).[16] 100 µL of extracts was applied in each well. DMSO was used as negative control and Vancomycin 30 µg as positive control then, incubated at 37°C for 24 hr. After that, the diameters of the zone of inhibition were measured. The experiment was repeated in triplicates.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration was performed by micro-broth dilution method to the plant which have high efficacy against MRSA.[17] Plants extracts were exposed to serial dilution by sterile MHB. Different plant extracts achieved through two-fold serial dilutions from columns 1-10 (from 5 mg to 0.0097 mg L). Column 11 contained 100 µL of MHB as negative control and column 12 contained 100 µL of diluted standardized inoculum as positive control. The standardized bacteria suspension (50 µL) was added to all wells, then incubated at 37°C for 24 hr. After that, 20 µL of resazurin (0.015%) was added to all wells and additional incubated for 2-4 hr for the observation of color change. Change color from blue to pink indicated the presence of MRSA, if no change in the color of dye indicates absence of MRSA.

Statistical analysis

Mean and standard deviation of means were determined to express data. Mega STAT was used to process the data and statement significant differences at $p<0.05$.

RESULTS

The antibacterial activity of plants extracts by different solvents, using the agar well diffusion method is shown in Table 2. In general, the negative control DMSO showed no inhibition effect on the growth of MRSA. On other hand, the positive control (antibiotic Vancomycin) induced inhibition zones 16.3 mm of MRSA growth. The average of zones of inhibition of extracts was significantly more with chloroform extracts of *Artemisia monosperma, Mentha longifolia*, and *Vernonia schimperi*. In addition, the average of zones of inhibition of extract of *Rumex nervosus* was significantly more in methanol extract. Whereas the alcoholic extracts of *Lavandula dentata, Leptadenia pyrotechnica, Commiphora myrrha* and *Achillea fragrantissima* have poor antibacterial activity against MRSA. The other plants: *Ocimmum basilicum, Ficus vasta, Ziziphus spina-christi, Caylusea hexagyna, Trichodesma trichodesmoides* var. *tomentosum* and *Anabasis articulate* have no inhibition zones were observed at this concentration (5 mg/0.5 mL). Also, *Ziziphus spina-christ* also has no inhibition zone. *Caylusea hexagyna* result disagree with

Table 1: Plants collected from different regions of Saudi Arabia.

Plants	Family	Parts used	Location
Artemisia monosperma	Asteraceae	Leaves	Collected from Nufud Al-thuwayrat, Algasim region (26°19'31.6"N 44°27'09.8"E.
Lavandula dentata	Lamiaceae	Leaves	Collected from Al- bin Jarada village, Al Namas city (19°04'00.9"N 42°07'14.4"E).
Mentha longifolia	Lamiaceae	Leaves	Collected from the village of Alkhudari, Tanuma city (19°00'12.5"N 42°10'32.5"E).
Ocimum basilicum	Lamiaceae	Leaves	Collected from khat center, 31 Km from Al- Namas city (19°.08104"N 42°.008"E).
Ficus vasta	Moraceae	Leaves	Collected from Al-sabt village, Tanuma city (18°57'04.8"N 42°10'18.5"E).
Ziziphus spina-christi	Rhamnaceae	Leaves	Collected from khat center, 31 Km from Al- Namas city (19°.08104"N 42°.008"E).
Caylusea hexagyna	Resedaceae	Stems	Collected from the village of Al-khudari, Tanuma city (19°00'12.5"N 42°10'32.5"E).
Rumex nervosus	Polygonaceae	Roots	Collected from Qantan valley, north of Tanuma city)19°00 56.1"N 42°07'35.7"E).
Leptadenia pyrotechnica	Asclepiadaceae	Stems	Collected from khat center, 31 Km from Al- Namas city (19°.08104"N 42°.008"E).
Commiphora myrrha	Burseraceae	Resins	Collected from Al-harth city, Jizan region (16°45'00.4"N 43°10'27.6"E).
Vernonia schimperi	Asteraceae	Aerial part	Collected from Fifa city (17.2541932, 43.0920545).
Trichodesma trichodesmoides var. *tomentosum*	Boraginaceae	Aerial part	Collected from Al-Namas city (19.0595990, 42.2297440).
Anabasis articulate	Amaranthaceae	Aerial part	Collected from Alqan, Tabuk city (29.1032810, 35.3974460).
Achillea fragrantissima	Asteraceae	Aerial part	Collected from Al-Jawf city (30.2813680, 38.2318460).

Table 2: Diameter of inhibition zones (mm) of different plant extracts. Mean of inhibition zones±SD of three replicates.

Diameter of inhibition zone (mm)			
Plants	**n-Hexane**	**Chloroform**	**Methanol**
Artemisia monosperma	10.6±4.56*	20.6±0.47***	20±0**
Lavandula dentata	10.6±0.47*	11±0.82**	11.3±0.94***
Mentha longifolia	10±0*	15±0***	14.6±0.47**
Rumex nervosus	0	10±0**	15±0***
Leptadenia pyrotechnica	9.5±0.47*	11.3±2.62**	11.6±0.47***
Commiphora myrrha	10±0	10±0	0
Achillea fragrantissima	11.6±0.47*	14±2.16***	13.6±2.96**
Vernonia schimperi	0	18.3±0.47***	14.3±0.47**
Ocimmum basilicum	No inhibition zones		
Ficus vasta			
Ziziphus spina-christi			
Caylusea hexagyna			
Trichodesma trichodesmoides var. *tomentosum*			
Anabasis articulate			
Positive control (Vancomycin 30 µg/mL)	16.3 ± 0.47		

Table 3: Minimum of inhibitory concentration mg/mL.

Plants	Chloroform	Methanol
Artemisia monosperma	1.25 mg/mL	-
Rumex nervosus	-	5 mg/mL
Mentha longifolia	2.5 mg/mL	-
Vernonia schimperi	5 mg/mL	-

the study, *Trichodesma trichodesmoides* var. *tomentosum* and *Anabasis articulate* also have no inhibition zones were observed. These results in these plants may because different solvent used or different parts of plants were used and may because these plants have no antibacterial activities at this concentration (5 mg/0.5 mL) (Table 3).

DISCUSSION

The antibacterial activity of *Artemisia monosperma* is like the results obtained in previous work which showed an antimicrobial activity of *Artemisia monosperma*.[16] In fact, it has been reported that *Artemisia monosperma* has bioactive constituent eriodyctiol-7-methyl ether was effective against *S. aureus*.[17] The results showed with *Lavandula dentata* has the same result in previous work which has antibacterial activity against MRSA with inhibition zone 11.7 mm.[18] This activity due to chemical compositions of the essential oil such as 1,8-cineole and *p*-cymen-8-ol.[16] Previous study on *Mentha longifolia* proposed that piperitenone oxide, menthone and other components are the major components of essential oil for antimicrobial activity.[18,19] *Rumex nervosus* has the same result in previous studied which showed that methanol extract of all parts of this plant have antibacterial activity. The results showed with *Leptadenia pyrotechnica* has the same work obtained in previous study.[10] The antibacteria activity of this plant may be attributed to the presence of terpenoids and flavonoids that triggered astringent and antimicrobial property.[20] The result showed with *Commiphora myrrha* has antibacterial activity, because its contains sesquiterpenoids which possess antibacterial, antifungal, and anesthetic activities.[21] The result showed with *Achillea fragrantissima* is similar with previous study that showed this plant have antibacterial activity against *S. aureus*.[22] *Ocimmum basilicum* has no inhibition zone at 5 mg/0.5 mL and in all solvents used for extraction disagree with other study that found *Ocimmum basilicum* at 100 mg/mL and extracted by ethanol have antibacterial activity against MRSA.[3,16]

CONCLUSION

The results shown in this study confirm that the plants extract of *Artemisia monosperma, Mentha longifolia, Vernonia schimperi* and *Rumex nervosu* which had high efficacy against MRSA infection, should be undergoes additional tests, to provide and explore compounds which have this function. Such simple and inexpensive alternatives to traditional treatment of bacterial infections may be deserved further rigorous investigation. We concluded that the plant extracts of *Artemisia monosperma, Mentha longifolia, Vernonia schimperi* and *Rumex nervosus* have anti MRSA activity that demonstrate promising anti-MRSA potential drug.

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ETHICAL APPROVAL

The study protocol was approved by the Ethics Committee of King Abdulaziz University, Jeddah, Saudi Arabia. The protocol was done according to the ethical guidelines of the 1975 Declaration of Helsinki.

CONFLICT OF INTEREST

The authors declare that there is conflict of interest.

AUTHORS CONTRIBUTIONS

TAK, SSM and EKB design protocol, SHA, TJA, SNS, SSY running experiments, TAA, SSM, EKB analyzed data and interpretations. ALL authors revise manuscript and approve it.

ABBREVIATIONS

MRSA: Methicillin-resistant *Staphylococcus aureus*; **MIC:** Minimum Inhibitory Concentration; **DMSO:** Dimethylsulfoxide.

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